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Using Emulsigen ® -D as Recent Adjuvant in Trivalent Foot and Mouth Disease Vaccine

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Abstract

The immunity and protective capability produced by vaccines can vary remarkably according to the kinds of adjuvant being used. Through this work three formulae of the inactivated trivalent FMD vaccine (O pan Asia, A Iran O5 , and SAT2 / EGY/2012) were prepared using different adjuvants including Emulsigen®-D; Montanid ISA 206 and Emulsigen®-D (ED) with aluminum hydroxide gel (ALOH). All of these vaccine formulae were found to be free from foreign contaminants and safe. Also, each vaccine formula was injected in a separate sheep group and serum samples were collected along 38-week post-vaccination for tracing of antibodies against FMDV serotypes by serum neutralization test (SNT) and enzyme-linked immune sorbent assay (ELISA).

Index terms— FMD, SNT, ELISA, emulsigen®-D; montanid ISA 206.

1 I. Introduction

oot-and-mouth disease (FMD) is a viral infectious disease that forms vesicles in the mouth and hooves of artiodactyls, such as pigs, cattle, sheep, and goats resulting in weight loss, reduced milk production and growth delays. The disease can be spread rapidly not only by the excrement of infected animals but also by contaminated feed, vehicles, and humans. Efforts directed to the eradication and prevention of FMD centering on stamping-out policies are controversial and the prevention, and control of the disease using vaccines have become areas of extreme interest Min-Eun et al 2016. Thus, the economic damage is substantial once an outbreak occurs. Therefore, FMD is subject to international regulations for the global trade of both livestock and their products Kitching 1999, Meyer and Knudsen 2001. The administration of vaccines is a highly effective method for preventing FMD.

The causative agent is the FMD virus which has seven serological types identified as O, A, C, SAT1, SAT2, SAT3, and Asia1 Doel and Baccarini 1981, Barnett and Carabin 2002. FMD is characterized by fever, lameness and vesicular lesions on the feet, tongue, snout, and teats with high morbidity and low mortality Satya 2009. The disease is enzootic in Egypt, with many outbreaks having been reported since 1950. The present serotypes of FMD virus in Egypt now are SAT2, A and O. Serotype O was lastly reported Aidaros 2002. Serotype A was firstly recorded in Egypt in 2006 through importation of live animals and resulted in sever clinical signs in cattle and buffaloes Abd El-Rahman et al 2006. The recent FMDV serotype introduction is the serotype SAT2 in 2012, also from the importation of live animals. All these serotypes were isolated and typed by Veterinary Serum and Vaccine Research Institute (VSVRI) and confirmed by World Reference Laboratory (WRL) for FMD, Pirbright Institute, United Kingdom Abd El-Aty et al 2013. Vaccination is the corner stone and effective method for preventing FMD. The selection of an appropriate adjuvant is the most important factor in determining the efficacy of potent vaccines to ensure a protective immunity enables susceptible animals to withstand the disease outbreaks Min-Eun et al 2016.

Emulsigen ® -D is an oil-in-water emulsion contains uniformly dispersed micron-size oil droplets, which ensure maximum emulsion stability and decreased viscosity. Micron-size oil droplets also increase the surface area

available to antigens, reducing the quantity of oil required in the final produced vaccine. Emulsigen ® -D reduces the undesirable side effects associated with other oil-in-water or water-in-oil adjuvants while eliciting a rapid and strong immune response Technologies M. Emulsigen ® -D Technical Bulletin 2012. Emulsigen ® -D as an adjuvant produces increased immunogenicity because it incorporates dimethyl-dioctadecyl ammonium bromide (DDA), which is a T-cell immune stimulator in Emulsigen ® . Its efficacy as an adjuvant was proved in Toxoplasma gondii and rabies Hiszczynska-Sawicka et al 2010 and Kaur et al 2010. According to Kaur et al 2010 the DDA contained in Emulsigen ® -D induces enhancement of immune responses by increasing the surface area of antigens in oil-in-water emulsions so that antigen spread slowly. Therefore, protection against Aujeszky's disease virus is increased when infected animals have been vaccinated with Emulsigen ® plus DDA. Also, aluminum compounds have been known to be the most frequently used adjuvant in veterinary vaccines Gupta 1998. These compounds have been found to induce memory cell responses and long-lasting protection when animals have been inoculated with vaccines, thereby enhancing immune reactions Rimaniol et al 2004. Among them, aluminum phosphate and aluminum hydroxide are the only adjuvants approved for routine use in humans because of their relatively low toxicity Li and Nookala 2007.

In this study, we evaluate comparatively the efficacy of experimental batches of FMD trivalent vaccine (including O pan Asia, A Iran O5 and SAT2 / EGY/2012) using various adjuvants as Emulsigen ® -D alone, and with Aluminium hydroxide gel and Montanide ISA 206 aiming to determine the best vaccine formula is having the optimum antigenicity and immunogenicity. The efficacy of prepared vaccine formulae will be tested in dairy sheep as one of the susceptible animal species for FMD.

2 II. Material and Methods

3 a) Ethical Approval

The experiment was carried out according to the protocol of the Institutional Animal Ethics Committee, and the authors had permission of the animal owners at the private farms.

4 b) FMD Virus Strains

Local Foot and Mouth disease virus serotypes O pan Asia, A Iran O5 and SAT2 / EGY/2012 propagated in Baby Hamster Kidney (BHK 21) cell line monolayer which was supplied by the Department of Foot and Mouth Diseases Research, Veterinary Serum and Vaccine Research Institute. The titer of the three serotypes was expressed as log 10 TCID 50 /ml as described by Reed and Muench 1938 and the complement fixation test was carried out according to Health Protection Agency 2009 These viruses were used for the preparation of trivalent inactivated vaccine as well as in serological tests.

5 c) Animals

6 i. Sheep

Twenty native breed sheep in a private farm free from FMD antibodies as screened by serum neutralization test were divided into four groups (5 animals/group).Each of 3 experimental FMD trivalent vaccines adjuvanted with Emulsigen ® -D, Emulsigen ® -D with ALOH, Montanide ISA 206, was inoculated as each in a sheep group keeping one group without vaccination as a negative control. The vaccine dose was 1.5 ml/animal inoculated subcutaneously where each dose contains 109 TCID50 of each type of Foot and mouth disease virus serotype.

7 ii. Suckling Baby Mice

Suckling Swiss baby mice, two to four days old, (Charles River Strain, USA) were used for testing the safety of the inactivated viruses according to OIE 2017.

8 d) Serum Samples

Serum samples were obtained from all sheep groups at the time of vaccination (zero time) then every week till four weeks, every two weeks for 16 weeks, every four week till 32 weeks post vaccination and lastly every two weeks till the end of the experiment (38 -weeks post vaccination). These samples were subjected for estimation of FMD antibodies in vaccinated animals using SNT and indirect ELISA.

9 e) Cell Culture

Baby Hamster kidney cell line (BHK21) was supplied by Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo using Eagle's medium supplemented with 8-10% bovine serum as described by Xuan et al 2011 and used for application of serum neutralization test, virus titration ,and vaccine preparation.

10 f) Virus Clarification and Inactivation

Each FMD virus serotype (O, A and SAT2) at the 7 th passage on BHK monolayer was treated with chloroform at a concentration of 1.5% (Volume / Volume) as a clarification method before inactivation. Inactivation was occurred using combination 1mM of BEI and 0.04% FA (BEI-FA) according to the method described by **??**arteling

11 h) Evaluation of the Prepared FMD Trivalent Vaccine i. Sterility and Safety Testing

The prepared vaccine batches were tested for their freedom of aerobic and anaerobic bacteria; fungal and mycoplasma contaminants where vaccines samples were cultured on thioglycolate broth, Sabouraud's, Nutrient agar; phenol dextrose media and mycoplasma medium. The safety of the prepared vaccines was done in baby mice according to OIE 2017.

12 i) The Potency of the Prepared Vaccines i. Evaluation of the Humeral Immune Response

Serum samples collected from the vaccinated sheep were tested for monitoring of the exhibited FMD antibody titers against the three serotypes by serum neutralization test (SNT) using the technique described by Ferreira 1976 and indirect enzyme-linked immune sorbent assay (ELISA) according to Voller et al 1976.

13 III. Results and Discussion

The control of FMD is dependent on the vaccination of susceptible animal species with inactivated whole virus vaccines Rodriguez and Grubman 2009. Vaccination with good quality FMD vaccines helps in the prevention of livestock production losses and reduces the overall incidence of the disease Hunter 1998. The selection of adjuvant in FMD vaccine formulation is important for both early and long-lasting immunity and protection. Hence, efforts are focused on developing adjuvant that can promote protective immunity through induction of enhanced and more durable antibody responses Dar et al 2013.

Attention is often directed to improve the potency of FMD vaccine aiming to provide the highest immune level in vaccinated animals to be able to withstand virus infection and accordingly avoid the suggested dramatic economic losses.

Emulsigen ® -D is a unique oil-in-water emulsion and contains uniformly dispersed micron-size oil droplets. These Micron-size oil droplets increase the surface area available to antigens, reducing the quantity of oil required in the final vaccine. Emulsigen ® -D incorporates dimethyl-dioctadecyl ammonium bromide (DDA) which is a T-cell immune stimulator. According to , the DDA contained in Emulsigen ® -D induces the enhancement of immune responses by increasing the surface area of antigens in oil-in-water emulsions so that antigens spread slowly. The use of ALOH gel in combination with oil is attributed as it is the most commonly used adjuvant in commercial vaccines Rimaniol et al 2004 and a previous report showed that AL induces Th2-type responses in animal models, facilitating the dissemination of antibodies from the injected region Gupta et al 1995 and Brewer et al 1996. Also, the gel was shown to play an important role in memory responses by inducing the differentiation of macrophages Min-Eun et al 2016. The combined components of oil and AL have been used to protect against rabies in bovines Reddy, and Srinivasan 1997. So in this study, we apply the use of Emulsigen ® -D and the use of ALOH gel in combination with oil as an adjuvant in foot and mouth disease vaccine and tracing the humeral immune response of sheep upon using these adjuvants.

This work deals with three prepared formulae of inactivated trivalent FMD vaccine (O pan Asia, A Iran O5 and SAT2 / EGY/2012) were prepared using three different adjuvants including Emulsigen ® -D; Montanid ISA 206 and Emulsigen ® -D with aluminum hydroxide gel The present obtained results revealed that all the prepared FMD trivalent vaccine formulae are free from foreign contaminants and safe inducing no abnormal post vaccination signs in vaccinated sheep in agreement with what recommended for such vaccine OIE 2017.

The antibody titer against the three serotypes (O, A and SAT2) were monitored in the serum samples using the serum neutralization and ELISA tests. Before vaccinating the different sheep groups, we ensure that all sheep involved in the experiment are free from antibody titer against the foot and mouth disease virus.

The results as tabulated in tables no. (1 & 2) and demonstrated by the figures (1-6) revealed that the onset of protective antibody titer was achieved early in the Emulsigen ® and Emulsigen ® with ALOH gel vaccinated groups as it starts at 2 nd week post vaccination while the onset of protective antibody titer in Montanide ISA 206 vaccinated group started at 3 rd week post-vaccination. Concerning the highest peak antibody titer values were induced by Emulsigen ® -D with aluminum hydroxide gel on 8 th -week post-vaccination (3.1, 3.2 & 3.21 log 10 for serotypes O, A & SAT-2 respectively); followed by Emulsigen ® -D on 10 th -week post-vaccination (2.9, 3.05 & 2.95 log 10 for type O, A & SAT-2 respectively) and then for Montanid ISA 206 on 12 th week post-vaccination (2.8; 2.9 and 2.6 log10 for type O, A & SAT-2 respectively) as evaluated by SNT. Concerning the duration of protective immunity against the three serotypes of FMDV included in the vaccine, the results revealed that the longest duration was achieved through the Emulsigen ® -D alone and with the ALOH adjuvanted vaccine as it lasts for 36 weeks postvaccination as recorded by the SNT values. The Montanide ISA 206 adjuvanted vaccine

group protective SNT antibody titer against the three serotypes lasts for 32 weeks post-vaccination. So from these results there is a two weeks protection duration difference between the different vaccinated groups.

ELISA results as a confirmatory test came in a parallel manner with those results obtained by SNT. From these results, it is clear that the use of Emulsigen ® -D adjuvant and, the addition of ALOH gel have a positive impact on the onset, peak and duration of protective immunity.

The previous results come in parallel with that obtained by Min-Eun et al 2014 as he mentioned that a high level of neutralizing antibodies in the ED + AL or ISA 201 groups exhibited a statistically significant difference from that in the ISA206 group. Regarding cellmediated immune responses, the ED and ED + AL vaccination groups exhibited statistically significant increases after antigen stimulation in both Th1 and Th2 cytokines, although they exhibited a low level of cytokines.Th1 reactivity was stronger in the ED + AL vaccination group than the ED-only vaccination group. Also, he found that a high level of neutralizing antibodies developed in a short period in the group of dairy goats inoculated with combined ED + AL, proving that Emulsigen ® -D in combination with aluminum hydroxide enhances the immune response in both pigs and dairy goats against foot and mouth disease virus.

In conclusion for the present work we found that the use of Emulsigen ® -D in sheep has an improvement immunogenicity effect over the use of the Montanide ISA 206 and also the use ALOH in combination potentiate the effects of ED adjuvants in the trivalent FMD vaccine. [1] [2] [3]

g) Formulation of the Prepared Experimental Vaccine Batches

The antigens were added to each of the following adjuvants:

1. Emulsigen ® -D (Emulsigen ® -D; MVP Technologies, NE, USA),
2. ISA 206 (Montanidetmisa 206 VG; SEPPIC, France)
3. Emulsigen ® -D with aluminum hydroxide gel (Rehydragel ® HPA; General Chemical, NJ, USA).

Figure 1:

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[2] Using Emulsigen®-D as Recent Adjuvant in Trivalent Foot and Mouth Disease Vaccine

[3] © 2018 Global JournalsUsing Emulsigen®-D as Recent Adjuvant in Trivalent Foot and Mouth Disease Vaccine

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WPV*	Mean FMD Serum Neutralizing Antibody Titers (Log10/Ml) in Sheep Group Vaccinated wi																			
0 1 2 3																				
4	1.8								1.91	1.76				1.9			1.96			1.95
6	1.95	2.16								1.91				2.32	2.39					2.21
8 10 12 14	2.16	2.43	2.45	2.72	2.8	2.9	2.74	2.92		2.25				2.56	2.79	2.9	3.05	2.91	2.8	2.64	2.66	2.49
										2.49												2.95
										2.6												2.9
										2.58												2.72
16 20 24 28	2.34	2.68	2.1	2.34	1.95	2.05	1.83	1.8		2.43				2.44	2.43	2.3	2.12	2.05	1.96	1.93	1.8	2.61
										2.21												2.44
										2.05												2.31
										1.94												2.24
30	1.62	1.59								1.6				1.78	1.69							2.05
32	1.5								1.53	1.49				1.66			1.6					1.82
34	1.35	1.46								1.32				1.62	1.55							1.7
36	1.21								1.3	1.05				1.57	1.53							1.6
38	1.05	0.95								0.86				1.51	1.34							1.48

*WPV= week-post-vaccination

[Note: G]

Figure 2: Table 1 :

2

Mean FMD ELISA Antibody Titers in Sheep Group Vaccinated with Trivalent FMD Vaccine Adjuvanated with

WPV*	Montanide ISA 206			Emulsigen ® D			Emulsigen ® D with ALOH Gel		
	O	A	SAT2	O	A	SAT2	O	A	SAT2
0	0.4	0.36	0.51	0.51	0.46	0.44	0.32	0.4	0.45
1	0.71	0.92	0.76	1.67	1.81	1.43	1.74	1.7	1.8
2	1.51	1.54	1.13	1.9	1.93	1.86	2	2.07	1.92
3	1.81	1.81	1.76	2.05	2.1	2	2.13	2.12	2.19
4	2.05	2.15	2.02	2.21	2.24	2.22	2.39	2.49	2.71
6	2.23	2.43	2.15	2.6	2.66	2.5	3.04	3	3.02
8	2.42	2.71	2.5	2.81	3.04	2.76	3.41	3.51	3.5
10	2.72	3.05	2.75	3.28	3.32	3.27	3.2	3.19	3.33
12	3.05	3.15	2.86	3.2	3.15	3.14	3.03	3.05	3.21
14	3	3.2	2.85	2.9	2.92	3	2.9	2.81	3.04
16	2.62	2.86	2.71	2.71	2.7	2.92	2.81	2.66	2.77
20	2.38	2.64	2.5	2.62	2.4	2.7	2.71	2.5	2.6
24	2.23	2.29	2.3	2.31	2.24	2.61	2.52	2.3	2.34
28	2.1	2.13	2.21	2.2	2.13	2.53	2.41	2.14	2.15
30	1.9	1.82	1.87	2.02	1.92	2.3	2.15	2.05	2.06
32	1.77	1.79	1.76	1.94	1.86	2.13	2.03	2	2.02
34	1.64	1.74	1.6	1.9	1.8	1.96	1.91	1.89	1.91
36	1.5	1.58	1.31	1.82	1.8	1.91	1.83	1.8	1.81
38	1.29	1.19	1.13	1.79	1.62	1.72	1.61	1.52	1.37

*[Note: *WPV= week-post-vaccinationFig. 1: Mean FMD Serum Neutralizing Antibody Titers (Log /Ml) against Serotype (O) in Sheep Group Vaccinated with Trivalent FMD Vaccine using different Adjuvants]*

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